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Evaluation of hydrated lime as a cubicle bedding material on the microbial count on teat skin and new intramammary infection

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In two experiments, the effect of applying hydrated lime as a cubicle bedding material on the microbial count on teat skin and new intramammary infection were evaluated. In experiment 1, dry dairy cows (n=60) were assigned to one of three cubicle bedding treatments for a 5 week period. The treatments applied were: Hydrated lime (HL), HL (50%) + Ground limestone (50%) (HL/GL) and GL. In experiment 2, two teat disinfectants products chlorhexidine (CH) and iodine (I) were applied to teats at milking in conjunction with two cubicle bedding materials with lactating cows (n=60) for a six-week period. The treatments applied were: HLCH; HLI; and GLI. The HL treatment had significantly more teats (P<0.001) with no *Staphylococcus* spp. or *Streptococcus* spp. bacteria present compared to GL. There were no differences observed between treatments for California Mastitis Test (CMT) score at calving or somatic cell count (SCC) post-calving. In experiment two, the HLI treatment tended (P<0.08) to have lower bulk milk SCC than the GLI. The average bulk milk SCC over the trial period was 68,000, 54,000 and 83,000 cells/mL for HLI, HLCH and GLI, respectively. The incidences of medium-term teat changes were numerically higher with HLI and there were no differences in the mean hyperkeratosis score between treatments. The mean teat hyperkeratosis scores on day 42 were 2.2, 2.1 and 2.1 for HLI, HLCH and GLI, respectively. The HLI treatment had lower levels of Staphylococcal and Streptococcal bacteria on teats compared to GLI (P<0.001). Hydrated lime could be successfully used as cubicle bedding material for dairy cows if used at the recommended rates with either chlorhexidine or iodine based teat disinfectants.

Keywords: Hydrated lime; microbial count; teat condition; somatic cell count

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Introduction

Mastitis represents a major economic cost to dairy farmers with losses of up €60 per cow for the average milk supplier (O'Brien 2008). As bulk tank milk somatic cell count (BMSCC) increased, from ≤100,000 to >400,000 cells/mL, the net farm profit generated decreased by €19,504 for the average Irish dairy farmer (Geary *et al.* 2012). Milk loss due to subclinical mastitis can also contribute to these overall farm losses (Hogeveen, Huijps and Lam 2011). *Staphylococcus aureus* is one of the major and more virulent pathogens that can cause mastitis infection and lactating cows are one of the main reservoirs of this species. Moreover, *S. aureus* colonisation of teat skin increased the risk of intramammary infection (IMI) (Myllys *et al.* 1993; Roberson *et al.* 1994). It was suggested that by minimising the exposure of teat ends to microorganisms, the rate of environmental infection levels were reduced (Smith, Todhunter and Schoenberger 1985). During a cow housing period, bedding materials such as sawdust, lime and sand are applied to cubicles to help to maintain a clean dry cubicle bed. By minimising pathogen growth within the bedding material, lower numbers of pathogens were transmitted onto the cow's teats, thereby reducing the possibility of IMI (Kudi, Bray and Niba 2009). However, materials of a fine particle size such as sawdust may support rapid growth of bacteria and can lead to high populations of bacteria on teats. Zdanowicz *et al.* (2004) demonstrated a correlation between environmental bacterial counts on teat ends with bacterial counts in sawdust bedding, which can create an environment for IMI (Hogan *et al.* 1999). However, some bedding materials like sand can minimise pathogen growth (Kudi *et al.* 2009). Hydrated lime (HL; calcium hydroxide) has been added to other bedding materials such as sawdust or shaving bedding to control bacterial populations (Fairchild *et al.* 1982). Increasing the pH of cubicle bedding can suppress bacterial growth (Kupprion *et al.* 2002). Hydrated lime is an alkaline compound that can create pH levels as high as 12.4. At levels greater than 12, the cell membranes of pathogens are considered destroyed (Chettri 2006). A one hundred-fold decrease in bacterial numbers has been reported when HL was added to recycled manure as a cubicle bedding (Hogan *et al.* 1999). However, anecdotal evidence suggests that the use of an iodine based post-milking teat disinfectant in conjunction with the use of hydrated lime can have a negative effect on teat condition. Long-term changes in teat condition generally occur over a period of 2 to 8 weeks (Neijenhuis *et al.* 2000). The condition observed is generally referred to as teat hyperkeratosis (Shearn and Hillerton 1996) and this condition can be exacerbated by disinfectants (Rasmussen 2004) or cold harsh weather (Timms, Ackermann and Kerlhi 1997). Medium-term changes to the teat barrel generally become visible within a few days or weeks of a management issue or environmental factors occurring (Ohnstad *et al.* 2007). These teat changes include petechial haemorrhages or larger hemorrhaging of the teat skin (Hillerton, Middleton and Shearn 2001). Other changes include cracking or chapping of the teat skin (Hillerton *et al.* 2001). There is little knowledge on the effect of using HL as the sole cubicle bedding material on bacterial numbers on teats, on teat condition and on IMI. The objectives of this study were 1) to establish if dry dairy cows housed in cubicles which were bedded using HL would have a reduced teat microbial count, lower new intramammary infections, have a better California Milk Test (CMT) result post-calving and have lower somatic cell counts (SCC)

for a three week period post-calving, compared to cows bedded with the commonly used ground limestone (GL) and 2) to establish if lactating dairy cows housed in cubicles which were bedded using HL combined with two contrasting teat disinfectant products would have less intramammary infections, lower SCC and more teat skin irritation as measured by teat-end hyperkeratosis and 'medium term teat changes', compared to cows bedded with the commonly used GL.

Materials and Methods

Experiment 1

Three cubicle bedding materials containing (i) Hydrated lime (HL), (ii) HL (50%) + Ground limestone (50%) (HL/GL) and (iii) GL were applied to three separate cubicle houses for a five week period. Sixty dry Holstein Friesian dairy cows were randomly assigned to one of three houses based on their expected calving date and lactation number.

Cubicle houses contained a central slatted passage with a single cubicle space allocation per cow and similar feed space per group. Cubicles were bedded once daily with sufficient material to leave a dry surface with no cubicle matt visible. Cows remained on the cubicle bedding material until calving (mean of 5 weeks dry period per cow) and then were managed outdoors on grass. Individual quarter milk samples were taken post-calving and classified using the CMT scores as follows: 1=200,000, 2=150,000 to 500,000, 3=400,000 to 1,500,000, 4=800,000 to 5,000,000, 5=>5,000,000.

Individual cow milk samples were collected using Weighall electronic milk meters (Dairymaster, Tralee, Co Kerry, Ireland) and analysed for SCC weekly for a three week period post-calving. Clinical cases were recorded during the five week period before calving and for a three week period post-calving. Milk samples for both Experiments 1 and 2 were examined using International Dairy Federation guidelines for microbiological analysis (IDF 1981).

Bacterial counts on teats

On the start date and once weekly thereafter, all teats from each cow in each group were swabbed using one sterile swab per cow (Cultiplast, Milan, Italy), before teat preparation for milking. The sterile swab was rubbed across the teat orifice and down the side of each teat avoiding contact with the udder hair or cows flank. Swabs were then placed in individual sterile bottles containing 5 mL of Tryptic Soy Broth (Becton-Dickinson, Sparks, USA). The broth was prepared in 500 mL volumes and autoclaved at 121 °C for 15 min, and then distributed into 5 mL aliquots in a Laminar Flow Cabinet. The sterile bottles containing the swabs were frozen (-20 °C) until analysed for the presence of *Staphylococcus* and *Streptococcus* bacteria. The swabs were streaked across two separate selective agars: Baird Parker (+ egg yolk emulsion 50 mL/l) (*Staphylococcus)* and Edwards (+ 6% sterile bovine or sheep blood) *(Streptococcus)*. Following incubation at 37 °C for 24 h, microbial counts for each pathogen type were manually estimated and assigned to one of four categories depending on bacterial numbers present. (0=no pathogen present, 1<10 (colony forming units per mL [cfu/mL]), 11<NS (too numerous to count), NS/IF (infinite numbers on the agar plate.)

Experiment 2

Hydrated lime was applied to cubicle beds in two houses and GL was applied to cubicle beds in a third house for a six-week period. Sixty lactating Holstein Friesian dairy cows were randomly assigned to one

of the three cubicle bedding treatments based on individual cow milk SCC (average of three previous weeks), lactation number, days in milk (120) and teat hyperkeratosis score. Cows assigned to one HL cubicle house and the GL house were pre and post sprayed at milking with an Iodine based (0.5%) teat disinfectant, HLI and GLI, respectively. Cows assigned to the second HL cubicle house were pre and post sprayed at milking with a chlorhexidine-based (Deosan Teatfoam, Diversey Hygiene Sales Limited, Jamestown Rd, Finglas, Dublin 11) teat disinfectant (HLCH). Pre-milking teat preparation included washing teats with running water, followed by the application of the relevant teat disinfectant and then drying with an individual paper towel for each cow. The average daily milk yield per cow over the test period was 23 kg per cow per day.

Treatment groups (n=20) were allocated separate cubicle rows which contained a slatted passageway with a single cubicle space allocation per cow, similar feed space per group and remained indoors for the duration of the experiment. All cubicles were fitted with rubber mats. Cubicles were bedded with the manufacturer specification rate for HL (170 g per cubicle) twice daily and this was applied to the back one-third of the cubicle. Higher application rates than that specified are considered unnecessary due to the drying properties of HL and the possibility of deterioration in teat condition. This HL product (White Rhino) is marketed as having a pH of 12.4 that can inhibit bacterial growth.

Cubicles bedded with GL received a more liberal application (approx 300 g per cubicle) twice daily, which is typical of rates normally applied for this product on farm. Ground limestone (Agrical, Nutribio Ltd, Tivoli Industrial Estate, and Cork, Ireland) is milled and crushed limestone rock which is used to soak up moisture on cubicle mats or concrete. The pH of this product is approximately 8–8.5.

Milk sample analysis

Individual quarter milk samples were taken at the start date and at day 42 and bacterial pathogens were identified as 0=no pathogens present, 1=*Staphylococcus aureus*, 2=Non-haemolytic *Staphylococcus*, 3=*Streptococcus dysgalactiae*, 4=*Streptococcus uberis*. Quarters with an SCC>500×10^6 cells/mL at the start date were considered sub-clinically infected and were excluded from this particular data set.

Individual cow milk samples were collected weekly using electronic milk meters (Dairymaster, Tralee, Co Kerry, Ireland) and analysed for SCC. Bulk milk samples with an SCC >500×10^6 cells/mL at the start date were considered sub-clinically infected and were excluded from this data set. The number of cows excluded for analysis was 2, 3 and 3 for HLI, HLCH and GLI, respectively. Individual quarter and bulk milk samples were analysed using a Somacount 300 (Bentley Instrument Company Limited, Dublin 12, Ireland).

Teat condition

All four teats of each cow were visually scored on three occasions (day 1, day 21 and day 42) by the same operator using a simplified classification system for the evaluation of hyperkeratosis (HK) in bovines (Neijenhuis *et al.* 2001). The classification scores were: Score1=normal teat-end orifice; Score2=slight smooth or broken ring of keratin; Score3=moderate raised smooth or broken ring of keratin; Score4=large raised smooth or broken ring of keratin. Teats were scored directly after milking, with the help of artificial light to illuminate teat ends and the score

totaled and averaged for each cow for the purposes of analysis.

Teat barrels were also inspected by the same operator at the start date and on five occasions thereafter for medium-term teat changes. Teats were scored for these conditions by visual assessment using a simple classification system as recommended by Mein *et al.* (2001) for assessing these conditions; Score1=Normal teat (smooth, soft, healthy skin), Score2=Dry skin (reddened or blue skin, flaky or rough skin with minimum cracking) and Score3= Open lesions (chapped, cracked).

On the start date and once weekly thereafter, all teats from cows in groups GLI and HLI were swabbed to measure bacterial numbers using the same technique and method of analysis as applied in experiment 1.

Statistical Analysis

Statistical analysis of data was performed using SAS software (SAS 2011). Cows were blocked in pairs according to lactation number and expected calving date for experiment 1 and on the average BM SCC (previous three weeks), days in milk and teat-end hyperkeratosis score for experiment 2. The statistical model was a mixed model with cows/blocks as the random effect and bedding treatment as the fixed effect and with repeated measures over time. Comparison was made between treatments for SCC and teat hyperkeratosis score at each measurement day and when data were pooled over the total measurement period. Interactions for time and treatment x time were also tested. A t-test was used to measure changes in quarter somatic cell count from day 1 to day 42 for each individual treatment. Differences in bacterial numbers observed on cow's teats were measured using Fisher's exact test. Where there was an effect of treatment a pair-wise comparison was conducted.

Results

Experiment 1

There were no differences observed in the number of quarters classified for a CMT score of 1 or >1 or for pathogens present in milk samples at calving between bedding treatments (Table 1). There were no significant differences observed for BMSCC between treatments at weeks 2, 3 and 4 post calving. The average BMSCCs over the trial period were 92,000, 60,000 and 74,000 cells/mL for HL, HL/GL and GL, respectively. There was one clinical case for each of HL and GL treatments over the four week lactating period.

There were no significant differences in teat bacterial numbers between treatments for experiment 1 and 2 on day 1. Numbers of both *Staphylococci* and *Streptococci* observed on teats reduced

Table 1. Number (%) of quarters with low (1) and high (≥2) California Mastitis Test scores at calving - Experiment 1

		California Mastitis Test[1] (%)	California Mastitis Test (%)	No. quarters with pathogens
Bedding material	No. of quarters	1	≥2	
Hydrated lime	55	48 (87)	7 (13)	1
Hydrated/Ground limestone	76	69 (91)	7 (9)	1
Ground limestone	67	66 (98)	1 (2)	3

[1]California Mastitis Test Score: 1=200,000 cells/mL and ≥2=150,000 to 5,000,000 cells/mL.

during the measurement period regardless of the cubicle bedding material applied. When swab counts for each measurement day were pooled, the HL treatment had more teats with no *Staphlococci* present compared to GL and had less teats with 'numerous' bacteria (11<NS) present compared to the HL/GL treatment (P<0.05) (Table 2). The HL bedding also had significantly more teats with no *Streptococci* present compared to GL (P<0.05) and HL/GL (P<0.001). The HL/GL bedding had less teats with 'numerous' bacteria present compared to the GL treatment (P<0.01).

Experiment 2

When the average SCC for quarters were compared, there were no significant differences between bedding treatments or within treatments at day 1 (start day) or at day 42 (finish day) (P>0.05). The average quarter SCC on day 1 was 29,000, 34,000 and 21,000 and on day 42 was 35,000, 41,000 and 39,000 cells/mL for HLI, HLCH and GLI treatments, respectively. Individual quarters with an SCC greater than one million cells per mL on day 42, were considered to be sub-clinically infected and not included in the average SCC data presented. The GLI treatment had a higher percentage of quarters with pathogens present in the milk sample on day 1 compared to the other two treatments (Table 3). At day 1 there were 1.3, 2.8 and 0 percent of quarters with a sub-clinical infection and at day 42 there were 1.4, 2.9 and 4.8 percent of quarters infected for HLI, HLCH and GLI,

Table 2. Differences in bacterial levels on teats (%) over a 6-week period - Experiment 1

	Hydrated lime	Hydrated lime + Ground limestone	Ground limestone	Hydrated lime	Hydrated lime + Ground limestone	Ground limestone
Colony forming units		***Staphylococcus* spp.**			***Streptococcus* spp.**	
0	30[a]	14[b]	17[b]	62[a]	42[b]	27[b]
1<10	43	53	48	13[a]	18[a]	41[b]
11<NS[1]	18	17	9	10[ab]	20[a]	4[b]
NS/IF[2]	9[a]	16[ab]	26[b]	14[a]	20[ab]	27[b]

Different letters as superscripts within the same row for each bacteria denote significant statistical differences among bedding treatments (P<0.05).
[1]11<NS=too numerous to count.
[2]NS/IF=infinite numbers.

Table 3. Milk quality and sub-clinical infection at day 1 and day 42 - Experiment 2

	Hydrated lime + Iodine		Hydrated lime + Chlorhexidine		Ground limestone + Iodine	
Somatic Cell Count (cells/mL)	**Day 1**	**Day 42**	**Day 1**	**Day 42**	**Day 1**	**Day 42**
> 200,000	1	3	6	7	0	5
< 100,000	87	91	88	88	93	94
< 20,000	79	68	70	65	80	71
Staphyloccus spp. or *Streptococcus* spp. present (%)	5	7	3	7	16	11
Sub-clinical infection (%)	1.3	1.4	2.8	2.9	0	4.8

respectively. The percentage of quarters with an SCC greater than 200,000 cells/mL increased (5%) with the GL bedding treatment compared to day 1. However, the percentage of quarters with an SCC>200,000 <100,000 or <20,000 cells/mL did not differ between all treatments on day 42.

Average bulk milk SCC (BMSCC) for the three bedding treatments are presented in Table 4. The average pre-experimental BMSCC was 57,000, 49,000 and 46,000 cells/mL for HLI, HLCH and GLI, respectively. The HLCH treatment had significantly lower BMSCC at week 5 ($P<0.05$) and tended to be lower ($P<0.08$) when all data were combined, compared to GL. The average BMSCC over the 6-week measurement period was 64,000, 56,000 and 84,000 cells/mL for HLI, HLCH and GLI, respectively.

The percentage of teats with 'medium term' teat changes at five observation dates is presented in Table 5. The changes in teat tissue observed in all cases were considered mild (score 2) and in the majority of cases were transient in nature. Changes observed included partial redness of the teat barrel and minor cracking of the teat skin. In two instances (HLCH) the observed poor teat condition persisted more than one week and the original instance only were included. There were no differences in the levels of medium term teat changes between HLI and HLCH treatments, with the GLI bedding treatment having the lowest number of teat tissue changes. The percentage of teats observed with changes over the trial period were 1.0, 1.8 and 0.3 for HLI, HLCH and GLI, respectively.

Mean teat hyperkeratosis scores for each bedding treatment at three observation dates are presented in Figure 1. The average teat end hyperkeratosis score did not differ between treatments at any measurement day ($P>0.05$, s.e.=0.13). Teat hyperkeratosis score increased from the

Table 4. Average bulk milk somatic cell count ('000) over a 6-week indoor period - Experiment 2

Week	Hydrated lime + Iodine	Hydrated lime + Chlorhexidine	Ground limestone + Iodine	s.e.	Significance
1	65	55	71	24.3	
2	51	49	74	23.9	
3	46	54	67	23.9	
4	72	63	91	24.5	
5	56[a]	48[a]	105[b]	24.0	*
6	93	66	94	24.7	
Combined data	64	56	84	12.5	0.08

Different superscript letters within a row denote statistical differences ($P<0.05$).

Table 5. Percentage of teats with 'medium term changes' to the teat barrel for three bedding treatments – Experiment 2

Day	Hydrated lime + Iodine	Hydrated lime + Chlorhexidine	Ground limestone + Iodine
7	2.5	1.3	1.3
14	1.3	5	0
21	0	0	0
35	0	1.3	0
42	1.3	1.3	0

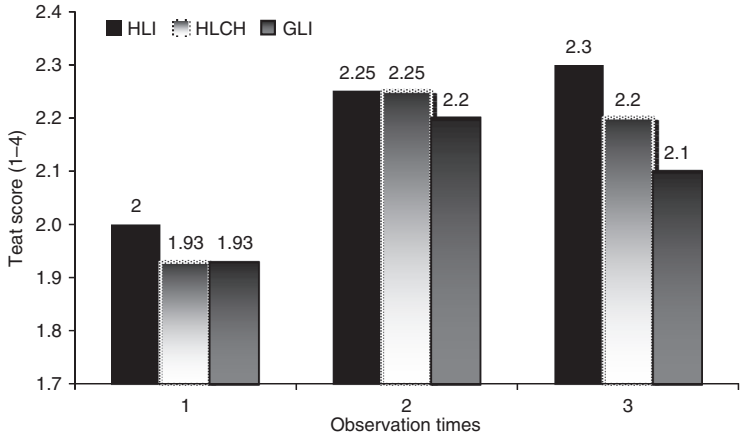

Figure 1. Average teat hyperkeratosis score on day 1, 21 and 42 – Experiment 2. HLI=Hydrated lime + Iodine, HLCH=Hydrated lime + Chlorhexidine, GLI=Ground limestone + Iodine.

first observation day compared to the subsequent two observation days (P<0.001), however, there was no time x treatment effect. The mean teat scores on day 42 were 2.2, 2.1 and 2.1 for HLI, HLCH and GLI, respectively.

The percentage of teats within four defined bacterial categories for treatment data pooled over five observation dates is presented in Table 6. The addition of HL to cubicle beds resulted in lower numbers of both *Staphylococci* and *Streptococci* on cows

Table 6. Percentage of teats with *Staphylococcus* spp. and *Streptococcus* spp. present within four defined bacterial categories over a five-week period - Experiment 2

Category	Hydrated lime + Iodine (n=100)	Ground limestone + Iodine (n=100)	Significance
Colony forming unit (cfu)/mL	*Staphylococcus* spp.		
0	60	25	***
1<10	26	40	*
11<NS[1]	5	17	**
NS/IF[2]	9	18	
	Streptococcus spp.		
0	69	35	***
1<10	18	42	***
11<NS	6	15	
NS/IF	7	8	

n=number of swab measurements per bacteria type.
[1]11<NS=too numerous to count.
[2]NS/IF=infinite numbers.

teats prior to teat preparation for milking at each observation day (Figure 2). The percentage of teats with no *Staphylococci* present was higher (P<0.001) for HLI (59%) compared to GLI (25%). The percentage of teats with bacterial counts within category 1≤10 was higher for GLI (36%) compared to HLI (15%). The GLI treatment had higher numbers (P<0.01) of teats within bacterial category '11<NS' and tended (P<0.09) to have a higher number within category 'NS/IF'. The percentage of teats with no *Streptococci* present was higher (P<0.001) for HLI (68%) compared to GLI (27%). The percentage of teats within the bacterial category 1≤10 was higher (P<0.001) for GLI (25%) compared to HLI (17%). The HLI treatment tended to have lower numbers (P<0.06) of teats within bacterial category 11<NS compared to the GLI treatment.

Discussion

The overall reduction in bacterial numbers for all treatments during the experimental periods probably indicates an effect of improved management for all cubicles during the test period. As cubicle bedding material becomes spoiled with faeces, the populations of bacteria in bedding can reach maximum levels 24 h after material is applied (Godkin 1999). In this present study, lime materials were applied to cubicle beds twice daily so potential bacterial growth was minimised.

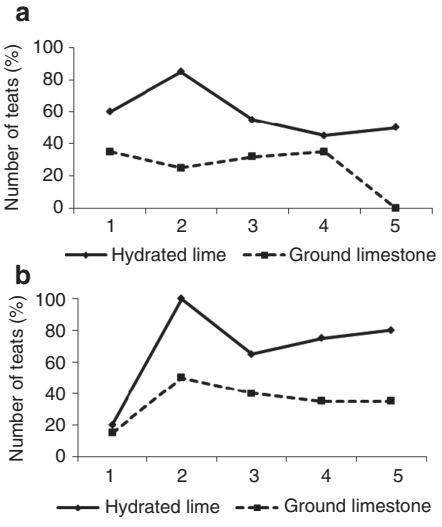

Figure 2. Percentage of teats with no detectable Staphylococci (a) and Streptococci (b) present over a five week period - Experiment 2.

A cubicle bedding material such as limestone is considered an ideal bedding material from a bacteriological point of view. The application of hydrated lime to organic cubicle bedding materials has previously been shown to lower total bacterial counts on cubicle beds by as much as 100-fold (Hogan *et al.* 1989b). Lower bacterial counts in bedding materials have also been associated with a decrease in the incidence of new infections (Hogan and Smith 2003) and in particular the incidences of new environmental infections (Hogan *et al.* 1999). The addition of hydrated lime directly to cubicle beds in this study resulted in lower numbers of both *Staphylococcus* spp. and *Streptococcus* spp. on the teats of both lactating and dry dairy cows. However, no significant reduction in new infection rates was observed. Previous studies by Chettri (2006) revealed that the daily application of hydrated lime in dairy cow free-stalls reduced the incidence of mastitis by approximately 45%; however, that study was conducted over a 12-month period. Furthermore, the average SCC was low at the trial start date in this study and new infection rates observed during both experiments were low, thus making it difficult to observe any significant differences between treatments. Furthermore, a higher rate of infections occur within the first 90 days of lactation (Hogan *et al.* 1989) and cows in this study were on average 120 DIM reducing the possibility

further of new intramammary infection. Satisfactory teat preparation (Gleeson *et al.* 2009) prior to milking in experiment 2 also nullified differences in teat bacterial numbers between treatments, as teat swabbing was conducted prior to teat preparation and this may also account for no differences in new intramammary infection between treatments. Very low bacterial levels on teats could be expected after teat disinfection as compared to levels taken before disinfection (Kristula *et al.* 2008). In Experiment one, the SCC levels recorded and the number of clinical cases observed during the period post calving were low for all bedding treatments. In this experiment, cows were grazed outdoors from calving and this management strategy may account for the low SCC and new infection levels recorded. Environmental factors can influence the microbial populations on teats ends (Rendos, Eberhart and Kesler 1975). Higher SCC levels have been reported during the months where cows are normally housed indoors (Nov to March) with SCC reducing during the summer period corresponding to when cows are grazed outdoors (Berry *et al.* 2006). The benefits of any carry over effect of the bedding material from the housed period pre-calving were not evident.

In Experiment two, an increase in the number of quarters with sub-clinical infections was observed for the non-hydrated lime bedding treatment compared to the start date and this may be partially due to the cows remaining indoors for the duration of this study. The percentage of GLI quarters with a sub-clinical infection was 1.9% higher than HLCH and 3.4% higher than HLI. However, this increase in sub-clinical infections during the trial period could be related to the high percentage of cows with bacteria present in quarters on day 1.

While all treatments had low SCC levels, the HLCH treatment had the lowest level at most tests days during the study. The HLCH treatment was also observed to have the lowest percentage of cows (9%) with a SCC>100k compared to the HLI (14%) and GLI (19%) during the study. This may indicate a positive benefit of disinfectant type rather than bedding material. Furthermore, the average quarter SCC was low at the trial start date and good management practices such as regular maintenance of the cubicle beds and teat preparation prior to milking, were important factors in maintaining low SCC levels and new infection rates for all treatments throughout the study. The milking process through improper milking time, hygiene and machine function can contribute to new infection rates when bacteria are present (National Mastitis Council 1996; Galton, Petersson and Merrill 1988). While the HL treatment had lower bacterial numbers on teats when presented for milking, all teats were prepared (washed, disinfected and dried with individual paper towels) prior to cluster application and this may have partially nullified the benefit of the bedding material as teat preparation has been shown to reduce teat bacterial numbers (Gleeson *et al.* 2009) and in particular environmental Streptococcal infections (Pankey *et al.* 1989). Should teat preparation be omitted/less rigorous, as in the case on many dairy farms in Ireland, differences in new infection rates may have been observed.

There were no differences in 'medium term' teat changes between the two hydrated lime treatments; however the GLI bedding treatment had a lower number of teat tissue changes during the study. Previous studies by Kristula *et al.* (2008) indicated that the application of HL at a rate of 0.5 kg per cubicle every 48 h caused an irritation to skin, udder and legs of cows,

with some lesions evident approximately 3 days after exposure to HL. There were no lesions to udder and legs in this study and only minor 'medium term teat changes', even though the application rate for HL was higher (340 g/day) compared to that applied by Kristula *et al.* (2008). The application of HL on four occasions instead of one in 48 h may also have influenced this outcome. Furthermore, it was suggested by Kristula *et al.* (2008) that stall designs that allow more manure to be deposited on the back end of the cubicle mattress may also exacerbate the irritation problem. Cubicles in this present study were cleaned down twice daily when the lime was applied.

The percentage of teat changes for all treatments were much lower than +5% which is an accepted level as an indicator of good milking machine function and operation (Hamann 1997). The average teat end hyperkeratosis score did not differ between treatments. The percentage of teats within score category 4 was similar (9%) for both the HLI and the GLI treatments and less than that suggested by Reinemann (2007) (20%), as an indicator of poor teat condition in a herd. The higher teat score observed over time in this study could be expected as hyperkeratosis score increases with stage of lactation (Neijenhuis *et al.* 2000). From a health and safety perspective when applying HL to cubicle beds it would be considered good practice to use a face mask as it tends to be dustier than GL.

Conclusions

The hydrated lime bedding treatment resulted in significantly less *Staphylococci* and *Streptococci* on teat skin compared to the ground limestone bedding treatment. Numerically lower levels of BMSCC and subclinical infections were observed with the hydrated lime treatments. A larger number of study animals and a longer test period may be necessary to show a significant effect of HL in terms of reduced new infection rates. Hydrated lime could be successfully used as cubicle bedding material for dairy cows if used at the recommended rates with either CH or I based teat disinfectants.

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